High Peak Estradiol Predicts Higher Miscarriage and Lower Live Birth Rates in High Responders Triggered with a GnRH Agonist in IVF/ICSI Cycles

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RESULTS: A total of 71 first cycles were analyzed. Rates of clinical pregnancy, live birth (LB), and total (clinical plus biochemical) miscarriage (MC) were 52%, 38%, and 25%, respectively. Mean peak estradiol (E2) and the number of oocytes retrieved were 3,701 pg/mL and 15.2, respectively. Peak E2 was significantly higher in those cycles resulting in clinical MC (p=0.003). After adjusting for age, basal follicle stimulating hormone, and the number of oocytes retrieved, elevated peak E2 remained associated with increased clinical MC (p=0.029) and trended toward a relationship with higher total MC (p=0.062). When peak E2 was treated as a binary variable based on the threshold value of >5,000 pg/mL, peak E2 above this value was associated with a higher rate of clinical MC (OR=16.14 with 95% CI 1.25–209.35, p=0.033) and total MC (OR=6.81 with 95% CI 1.12–41.54, p=0.037), as well as a lower LB rate (OR=0.095 with 95% CI 0.01–0.90, p=0.041).

CONCLUSION: Clinicians should recognize most IVF/ICSI patients triggered with a GnRH agonist as inherently in danger of excessively high serum E2 and avoid peak levels >5,000 pg/mL in order to avoid higher MC and lower LB rates. (J Reprod Med 2015;60:463–470)

Keywords: agonists, assisted reproductive techniques, assisted reproductive technology, con-

Ovarian hyperstimulation syndrome (OHSS) is a well-recognized and potentially fatal iatrogenic complication of controlled ovarian hyperstimulation (COH) during in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles. While its true incidence is probably vastly underestimated due to inconsistent reporting, a protocol development has arisen in the past decade allowing for a paradigm shift in the treatment of high-risk patients and a virtual elimination of this serious complication.1

Triggering the induction of final oocyte maturation with a gonadotropin-releasing hormone agonist (GnRHa) instead of human chorionic gonadotropin (hCG) was first proposed over 20 years ago as a means to decrease the burden of OHSS.2 While it achieves this end with great efficiency, subsequent studies demonstrated poorer pregnancy outcomes attributable to defective hormone production from GnRHa-induced luteolysis.3-5 However, as a subject of significant investigative interest during the past several years, newer studies promoting further protocol changes have brought about greatly improved pregnancy outcomes while maintaining a very low incidence of OHSS.6-12 As a result, clinicians have become more comfortable using GnRHa trigger, given their propensity for a high response, patients triggered with a GnRHa, given their propensity for a high response, may be quietly in danger of a worse prognosis than would otherwise be expected.

Materials and Methods
Study Design and Participants
This was a retrospective cohort study conducted at the Duke Fertility Center. Study approval was granted by the Duke University Institutional Review Board. The records of all patients who underwent fresh, autologous IVF/ICSI using a GnRH antagonist protocol and a GnRHa trigger for oocyte maturation from January 2010 to December 2012 were reviewed. Seventy-one first cycles were included for analysis. All patients were deemed high risk for OHSS based on age, ovarian reserve testing, peak serum E2, and/or >13 follicles measuring >11 mm in diameter on the day of trigger.23

Treatment Protocol
The treatment and LPS protocols used were previously described by Engmann et al.12 Baseline transvaginal ultrasound was performed following 2–3 weeks of oral contraceptive pill suppression. On menstrual cycle day 1–2, a daily subcutaneous injection of recombinant follicle stimulating hormone (rFSH) (Follistim, Merck, Whitehouse Station, New Jersey) was initiated. The usual starting dose of 100–225 IU was based on patient age, body mass index, antral follicle count, and anti-Müllerian hormone level (available in 63/71 patients). Serial ultrasounds and serum E2 measurements were performed to assess response to stimulation and adjust medication dose at the discretion of the treating physician. When the lead follicle reached a mean diameter of 13 mm and/or the serum E2 exceeded 350 pg/mL, a daily subcutaneous injection of recombinant follicle stimulating hormone (rFSH) (Follistim, Merck, Whitehouse Station, New Jersey) was initiated. The usual starting dose of 100–225 IU was based on patient age, body mass index, antral follicle count, and anti-Müllerian hormone level (available in 63/71 patients). Serial ultrasounds and serum E2 measurements were performed to assess response to stimulation and adjust medication dose at the discretion of the treating physician. When the lead follicle reached a mean diameter of 13 mm and/or the serum E2 exceeded 350 pg/mL, a daily subcutaneous injection of 250 µg of the GnRH antagonist ganirelix acetate (Ganirelix, Merck) was added to prevent premature ovulation. These were continued until the day of oocyte maturation induction. When ≥3 follicles reached ≥17 mm in mean diameter, 1 mg of leuprolide acetate (Lupron, TAP Pharmaceuticals, Lake Forest, Illinois) was administered subcutaneously on the day of trigger.
E2, LH, and P levels were measured the following morning to ensure adequate surge. Transvaginal, ultrasound-guided oocyte retrieval was performed approximately 36 hours after trigger injection.

**Luteal Phase Supplementation**
All patients received 50 mg intramuscular (IM) P in oil daily and 0.3 mg E2 patches (Vivelle-Dot, Novartis Pharmaceuticals, East Hanover, New Jersey) every other day beginning on the day after oocyte retrieval and continuing until either 10 weeks of gestation or a negative pregnancy test. Serum E2 and P were measured 3 and 7 days after oocyte retrieval and then weekly until 10 weeks of gestation. To maintain serum E2 above 200 pg/mL, either patches were increased up to 0.4 mg every other day or 2 mg of twice-daily oral, micronized E2 (Estrace, Bristol-Meyers, New York, New York) was added, as necessary. Similarly, IM P was increased to 75 mg daily, as needed, to keep serum levels above 20 ng/mL.24

**Outcome Variables**
The primary outcome was live birth (LB). Secondary outcomes included rates of clinical pregnancy (CP), clinical miscarriage (MC), total MC, and moderate to severe OHSS. Clinical pregnancy was defined as the presence of an intrauterine gestational sac (GS) at 6–7 weeks’ gestation coincident with a rising serum β-hCG level. Clinical MC was defined as pregnancy loss after ultrasound visualization of an intrauterine GS. Total MC included clinical plus biochemical MC, the latter defined as pregnancy loss prior to ultrasound identification of a GS.

**Data Analysis**
The SAS statistical package (Release 9.3, SAS Institute, Cary, North Carolina) was used for statistical analysis. Summary statistics for baseline and cycle variables were computed and are presented as means (standard deviation [SD]). Univariable analysis to compare the equivalence of each clinical variable between outcome statuses was performed using two-sample t tests for continuous variables and χ² tests for categorical variables. Multiple logistic regression modeling was performed for each variable of interest with covariate adjustment for patient age, basal FSH, and the number of oocytes retrieved. Estimated odds ratios (ORs) and their 95% confidence intervals (CIs) are reported. A nominal significance level (p<0.05) was used to declare statistical significance.

**Results**
Baseline and cycle variables are shown in Table I. The mean age was 33.4 years. The average anti-Müllerian hormone level and antral follicle count were 5.0 ng/mL and 21.2, respectively. All patients demonstrated adequate surge after trigger. The rates of CP, clinical MC, total MC, and LB were 52.1%, 14.1%, 25.4%, and 38%, respectively. The rate of twin gestation was 15.5%, and none of these pregnancies ended in miscarriage. There were no cases of high-order multiples or OHSS.

The mean peak E2 and number of oocytes

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>71</td>
<td>33.41 (3.94)</td>
<td>26</td>
<td>42</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>71</td>
<td>27.12 (6.72)</td>
<td>18.7</td>
<td>49</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
<td>63</td>
<td>5.01 (3.59)</td>
<td>0.47</td>
<td>19</td>
</tr>
<tr>
<td>AFC (n)</td>
<td>71</td>
<td>21.24 (10.39)</td>
<td>3</td>
<td>51</td>
</tr>
<tr>
<td>D3 FSH (mIU/mL)</td>
<td>47</td>
<td>7.16 (1.99)</td>
<td>0.6</td>
<td>11.8</td>
</tr>
<tr>
<td>Days of stimulation (n)</td>
<td>71</td>
<td>9.81 (1.46)</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Peak E2 (pg/mL)</td>
<td>69</td>
<td>3,700.65 (1,669.47)</td>
<td>1,042</td>
<td>7,208</td>
</tr>
<tr>
<td>Posttrigger P4 (ng/mL)</td>
<td>69</td>
<td>9.67 (4.91)</td>
<td>1.8</td>
<td>23</td>
</tr>
<tr>
<td>Posttrigger LH (IU/L)</td>
<td>68</td>
<td>37.75 (20.62)</td>
<td>9.7</td>
<td>98.1</td>
</tr>
<tr>
<td>Oocytes retrieved (n)</td>
<td>71</td>
<td>15.18 (6.90)</td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>2PN (n)</td>
<td>71</td>
<td>8.63 (4.34)</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Cleaved (n)</td>
<td>71</td>
<td>8.52 (4.27)</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Transferred (n)</td>
<td>71</td>
<td>1.99 (0.43)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Cryopreserved (n)</td>
<td>71</td>
<td>5.45 (4.20)</td>
<td>0</td>
<td>26</td>
</tr>
</tbody>
</table>

BMI = body mass index, AMH = anti-Müllerian hormone, AFC = antral follicle count, D3 FSH = day 3 follicle stimulating hormone, E2 = estradiol, posttrigger P4 = progesterone on day after trigger, posttrigger LH = luteinizing hormone on day after trigger, 2PN = two pronuclear zygote.
retrieved were 3,701 pg/mL and 15.2, respectively. Pregnancy outcomes per peak E2 level for the entire cohort are shown in Table II. While not statistically significant, peak E2 was lower in pregnancies resulting in LB. It was, however, significantly higher in those resulting in clinical MC (p = 0.003). Post-trigger LH and P4 were not related to the investigated outcomes (Table III). After adjusting for age, basal FSH, and the number of oocytes retrieved in the multiple logistic regression model, peak E2 remained associated with increased clinical MC (p = 0.029) (Table III) and trended towards a relationship with higher total MC (p = 0.062).

We then examined the frequency of clinical MC for each 1,000 pg/mL peak E2 interval (Figure 1). Due to the abrupt increase in clinical MC above 5,000 pg/mL, we chose this value as the threshold around which to dichotomize the data (peak E2 status). That is, clinical MC rate ranged from approximately 6–14% in each of the five 1,000 pg/mL groups below 5,000 pg/mL but increased to 33% (5/15 patients) when peak E2 reached ≥5,000 pg/mL. Once again controlling for age, basal FSH, and the number of oocytes retrieved, peak E2 ≥5,000 pg/mL was associated with significantly higher rates of clinical MC (OR 16.14 with 95% CI 1.25–209.15, p = 0.033) and total MC (OR 6.81 with 95% CI 1.12–41.54, p = 0.037), as well as fewer LBs (OR 0.010 with 95% CI 0.01–0.90, p = 0.041) (Table IV).

Discussion

Our findings demonstrate that extremely supraphysiologic E2 levels appear to be associated with worse pregnancy outcomes in fresh, autologous, GnRH antagonist IVF/ICSI cycles when using a GnRHa trigger. After adjusting for confounders, fewer LBs and more MCs were observed with elevated peak E2, especially when it exceeded 5,000 pg/mL. These results contribute to a recent focus in the literature attempting to clarify defective endometrial receptivity from supraphysiologic hormone levels in fresh cycles as the causative mechanism for poorer obstetric outcomes. Indeed this association may be even more pronounced in cycles using a GnRHa trigger, owing to the added corpora lutea (CL) dysfunction from the agonist, and there is currently a paucity of literature on the combination of these two topics. Our study suggests that, similar to IVF/ICSI cycles using hCG trigger, optimal pregnancy outcomes are achieved in GnRHa-triggered cycles either by avoiding excessively high E2 levels during fresh cycles or by cryopreserving all embryos for subsequent frozen embryo transfer (FET). Moreover, it seems imperative to recognize this cohort of patients as inherently at risk for this scenario given their tendency for high responders.

hCG has historically been the therapeutic means to replace endogenous LH to induce oocyte maturation and ovulation in IVF/ICSI cycles. Due to their similar structure and biological activity, hCG and LH act on the same receptor. However, the half-life of LH is approximately 60 minutes, whereas that of hCG is >24 hours. Thus, hCG produces a much more sustained effect on the CL, predisposing normal and high responders to a greater risk of OHSS. Specifically, LH receptor stimulation on the ovarian theca cells stimulates production of androstenedione, which then diffuses into the granulosa cells and serves as the primary precursor for E2 production. It is the exaggeration of this process, particularly in high responders, that contributes to the pathogenesis of OHSS and altered endometrial receptivity.

With the advent of GnRH antagonist protocols in the late 1990s it became possible to induce oocyte maturation with a GnRHa. Doing so causes an initial surge of LH and FSH followed by pituitary suppression with continued use. But the rise and fall of this “flare” is much more abbreviated than in a natural cycle, and thus the total amount of gonadotropin released from the pituitary after

<table>
<thead>
<tr>
<th>Table II</th>
<th>Pregnancy Outcomes per Peak Estradiol Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome</td>
<td>&lt;2,000 n=12</td>
</tr>
<tr>
<td>+ Pregnancy test (%)</td>
<td>50</td>
</tr>
<tr>
<td>Clinical pregnancy (%)</td>
<td>17</td>
</tr>
<tr>
<td>Live birth (%)</td>
<td>17</td>
</tr>
<tr>
<td>Miscarriage (%)</td>
<td>17</td>
</tr>
</tbody>
</table>
GnRH is significantly less. One potential benefit of GnRHa trigger, however, is the simultaneous release of FSH, akin to the natural cycle. FSH has been shown to induce LH receptor formation on granulosa cells and promote nuclear maturation and cumulus expansion, which may explain why some studies using GnRHa trigger have reported a significantly higher number of mature oocytes compared to hCG trigger.

In 1988 Itskovitz was the first to propose the use of GnRHa for oocyte maturation as a way to decrease the incidence of OHSS in IVF cycles. In the past decade over a dozen randomized controlled trials (RCTs) have proven the efficacy of GnRHa in eliminating OHSS. As alluded to above, during the same time period multiple well-designed studies demonstrated no difference, or even an increase, in the rate of metaphase II oocytes, as well as no detriment to fertilization or embryo quality with GnRHa trigger. Despite these apparent accomplishments, widespread use of GnRHa trigger has been met with resistance, largely due to several initial reports of poorer implantation, lower ongoing pregnancy rates, and higher pregnancy loss rates in normal responders using standard LPS.

In Table III, we compare hormone variables between pregnancy outcomes.

<table>
<thead>
<tr>
<th>Category (N)</th>
<th>Live birth Mean (SD)</th>
<th>Clinical miscarriage Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LB (27)</td>
<td>No LB (44)</td>
</tr>
<tr>
<td>Peak E2 (pg/mL)</td>
<td>3,310 (1,998)</td>
<td>3,952 (1,956)</td>
</tr>
<tr>
<td>Posttrigger LH (IU/L)</td>
<td>38.8 (21.3)</td>
<td>37.1 (20.4)</td>
</tr>
<tr>
<td>Posttrigger P4 (ng/mL)</td>
<td>10.3 (5.9)</td>
<td>9.3 (4.2)</td>
</tr>
</tbody>
</table>

SD = standard deviation, E2 = estradiol, LH = luteinizing hormone, P4 = progesterone.

*p values derived from t test.

Table III Comparison of Hormone Variables Between Pregnancy Outcomes

The luteolytic property of the GnRHa, one way or another, is key to preserving pregnancy potential after GnRHa trigger in autologous, fresh cycles. Current recommendations from the Copenhagen GnRH Agonist Triggering Workshop Group expand upon this idea as they make recommendations for the use of GnRHa trigger in several patient populations. According to their 2011 publication, for all patients, good oocyte quality can be achieved with GnRHa trigger while practically eradicating the risk of OHSS. They advocate its universal use in oocyte donors, highlighting the added benefits of a shorter luteal phase and reduction in ovarian size and pelvic free fluid after oocyte retrieval. Finally, while the optimal LPS regimen is yet to be fully elucidated, ongoing pregnancy rates similar to hCG trigger can be achieved in one of two ways: (1) by adding one or more injections of low-dose hCG or rLH at the time of trigger (“dual trigger”) and/or in the luteal phase, in addition to “modified” E2 and P4 supplementation, or (2) by freezing all embryos and transferring them after thaw in a subsequent programmed cycle.  

![Figure 1](https://example.com/fig1.png)

Figure 1 Clinical miscarriage frequency per 1,000 pg/mL peak E2 interval. CM = clinical miscarriage, E2 = estradiol.
At present, the relationship between the supra-physiologic hormonal milieu of fresh IVF cycles and endometrial receptivity continues to be debated. Many investigators have examined the issue over the past 25 years. Despite an assortment of differing study designs and populations that ultimately make definitive conclusions elusive, when considered collectively, the data seem to suggest an adverse endometrial effect of very elevated E2. Forman et al demonstrated significantly lower implantation and pregnancy rates in cycles with high E2.13 Simon et al faulted altered endometrial receptivity after similar findings in normal and high responders with peak E2 >2,500 pg/mL, despite no difference in oocyte number or embryo quality.14 Another study by the same author concluded improved receptivity in high responders by using an rFSH step-down protocol to achieve a lower peak E2.15 Similar conclusions were drawn from two studies that found inferior pregnancy rates in fresh but not in frozen/thaw cycles when peak E2 exceeded 5,000 pg/mL.16,17 A 2010 study of 455 fresh IVF cycles using hCG trigger suggested an optimal peak E2 threshold of 4,000 pg/mL for women <38 years of age and 3,000 pg/mL for those ≥38.18 Moreover, endometrial gene expression, including that for E2 and P receptors, has been shown to be significantly altered in stimulated cycles, indicating a state of premature endometrial advancement compared to natural cycles.19-21

Perhaps the most compelling evidence in support of the notion of defective endometrial receptivity associated with supra-physiologic hormone levels in stimulated cycles is the growing number of studies showing better outcomes in freeze-all/thaw cycles. In separate RCTs investigating normal and high responders, Shapiro et al demonstrated improved clinical and ongoing pregnancy rates after elective FET.35,36 In another elegant study from the same group, 93 pairs of fresh and frozen/thawed blastocysts were matched on embryo parameters and patient age.37 Single blastocyst transfer revealed a significantly better PR in the frozen/thaw group for day-6 blasts (54.3% vs. 17.1% in the fresh group, p<0.001). This resulted in a significantly higher ongoing PR in the frozen/thaw group as compared to the fresh (55.9% vs. 26.9%, respectively, p<0.001). The authors point toward impaired embryo implantation from altered endometrial receptivity in the setting of recent ovarian stimulation. They highlight their findings as support for embryo-endometrium asynchrony since the more slowly-developing blasts (day 6) appeared to be most affected; i.e., the slower-growing embryos may miss a more advanced or narrow implantation window after recent COS.

Finally, the theory of impaired endometrial receptivity has gone so far as to surface in literature regarding obstetric and perinatal outcomes. A 2012 metaanalysis of 11 observational studies comparing singleton pregnancies in fresh versus frozen/thaw cycles demonstrated better outcomes in the latter group.38 These include significantly lower relative risks of antepartum hemorrhage, preterm birth, small-for-gestational-age infants, low birth weight, and perinatal mortality. Once again, the authors postulate that very high steroid hormone levels during COH result in endometrial advancement causing asynchrony with early embryo development that ultimately leads to abnormal placentation that contributes to these complications.

The results of the present study support a role for altered endometrial receptivity to explain poorer pregnancy outcomes in high responders whose cycles have resulted in very high peak E2 levels. Weaknesses of the study include its retrospective design and small sample size. Therefore, estimates of the outcomes of interest are based on relatively few observations, and type 1 error cannot be excluded. Furthermore, CP, LB, and MC, despite being clinically significant outcomes, are statistically correlated. By repeating them in a multivariate analysis, we cannot exclude the possibility of finding associations by chance. In addition, selection bias may exist from heterogeneity in medication dosing and patient diagnosis (for example, the cohort included some patients with polycystic ovary syndrome). However, our inclusion of only GnRH antagonist cycles using a GnRHa for oocyte maturation makes our findings unique to the vast majority of published data on the subject and draws attention to a particularly vulnerable subgroup of patients.

In an era when comfort with GnRHa trigger is becoming more common thanks to improvements in LPS, including the use of “dual trigger,” the continuing trend of evidence linking the supra-physiologic hormone milieu of COH to impaired endometrial receptivity deserves added reflection. The use of GnRHa trigger itself, in fresh, autologous cycles, should probably serve as an immediate red flag because it identifies those patients most likely to demonstrate an exaggerated response to stimulation. With the realization that the inci-
dence of moderate to severe OHSS after GnRHa trigger is extremely low, less importance is often placed on peak E2. That is, agonist trigger prevents OHSS even with extremely high E2 levels. Thus, it becomes easy to aggressively stimulate patients with less regard to rising E2, knowing the agonist trigger is such an effective guarantee against OHSS. In this way, the attempt to prevent one ill effect directly opens the door for another. Our findings suggest that such a practice is a mistake.

The importance of this observation may be further underestimated when one considers the luteolytic property of the agonist, as it seems plausible that this may have an additive negative effect on endometrial receptivity. The detriment from embryo/endometrium asynchrony due to high hormone levels may be compounded by deficient E2 and P production from the CL, creating an even more fragile environment for proper implantation.

In conclusion, very high levels of peak E2 in high responders triggered with a GnRHa should be avoided to maximize endometrial receptivity and improve pregnancy outcomes, irrespective of OHSS prevention. Clinicians should recognize this patient population as inherently in danger of this scenario and minimize the occurrence of peak E2 levels >5,000 pg/mL. Well-designed, prospective trials are needed to shed further light on the concept of endometrial receptivity in IVF/ICSI cycles using a GnRHa trigger.

Acknowledgments

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References


Table IV Results of Multivariate Logistic Regression Analysis for Each Clinical Outcome Using Dichotomized Peak E2 Status (_>5,000 pg/ml Compared to <5,000 pg/ml), with Adjusted Covariates

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Peak E2 status</th>
<th>Covariates (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>0.53</td>
<td>0.10–2.66</td>
</tr>
<tr>
<td>Clinical miscarriage</td>
<td>16.14</td>
<td>1.23–209.35</td>
</tr>
<tr>
<td>Total miscarriage</td>
<td>6.81</td>
<td>1.12–41.54</td>
</tr>
<tr>
<td>Live birth</td>
<td>0.10</td>
<td>0.01–0.90</td>
</tr>
</tbody>
</table>

OR = odds ratio.


